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Host-parasite relationship of Chalara quercina and species of quercus

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NOTE TO USERS

HOST-PARASITE RELATIONSHIP OF *CHALARA QUERCINA* AND SPECIES OF QUERCUS

by

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TABLE OF CONTENTS

INTRODUCTION

During the past five years oak wilt has become increasingly important in the upper Mississippi valley. Wilt has been reported from Iowa, Illinois, Minnesota, Missouri and Wisconsin (5, 22, 36). Conservationists and foresters recognize it as the most serious problem in oak culture in Iowa. Although the disease spreads slowly, large numbers of oaks are killed each year because of the tremendous number of infected areas. In farm wood-lots in northeastern Iowa sufficient trees were killed in 1945 (14) to constitute almost 70 per cent of the potential annual red oak growth in those areas.

The disease is most serious on trees of the red oak group. Large, mature trees may be dead within 4 to 6 weeks after symptoms first appear. Red oaks infected in late summer or fall may leaf out in the following spring, but develop typical symptoms and die within a few weeks. No red oak has ever been observed to recover from the disease. White and bur oaks usually die more slowly. Affected branches die, producing numerous stagheads, but diseased trees may live for several years following initial infection.

Little is known about oak wilt beyond the fact that it is caused by the fungus Chalara quercina Henry. No method has been suggested that would account for the invasion and destruction of a 50 to 60-foot red oak tree within a period of a few weeks. In order to learn more about the action of the pathogen and to develop measures for alleviating its injury and reducing its spread, a series of field, greenhouse and laboratory tests

were made on the disease. Some of the observations on the host-parasite relations are reported in this thesis.

PERTINENT LITERATURE

Oak Wilt

Death of oak trees in the upper Mississippi valley has been observed for many years. Warder (47) in 1880 described a general diseased condition of black oaks in Wisconsin. Tiemann (44) in 1927 reported the death of large numbers of oaks in the lake states region. Both the symptoms and pattern of spread described by Tiemann are similar to those now associated with oak wilt. Photographs of trees dying at McGregor, Iowa, in 1932, and subsequent isolation of the oak wilt organism since 1944 from centers adjacent to those photographed, leave no doubt that oak wilt was present in northeastern Iowa more than 15 years ago. Woodsmen trained to recognize oak wilt report that trees with similar symptoms were dying more than 30 years ago.

The fungal nature of oak wilt was first reported in 1942 by investigators at the University of Wisconsin (50). Prior to that time the progressive wilting, bronzing and defoliation of oaks had been attributed to insects, drought and various other agencies. Henry (20) in 1944 described the causal organism of oak wilt and named it Chalara quercina H. The pathogen was isolated from wilting oaks in Wisconsin, Minnesota, Iowa and Illinois by Henry et al. (22). In Wisconsin the most severe infection was noted in the southern and western part of the state. The disease has been observed generally over the state of Iowa (12), in residential districts of St. Louis (8), and in other localities in Missouri (10).

No correlation was found between site, kind of soil, nutrients present or soil reaction and the incidence of oak wilt in Wisconsin (22). In Iowa the disease has been observed in state park and forest areas, farm wood lots and on private lots in residential districts (14).

Apparently all species of oak are susceptible to oak wilt. Henry et al. (22) reported the isolation of Chalara quercina from naturally infected Q. alba, Q. macrocarpa, Q. borealis, Q. coccinea and Q. velutina. Natural infection was observed in Iowa on all of the above species except Q. coccinea, which is not native to the state, and on Q. ellipsoidalis, Q. imbricaria, Q. marilandica and Q. stellata in 1944 (36). In 1945 Dietz (11) reported successful greenhouse inoculation of Q. alba, Q. borealis, Q. ellipsoidalis, Q. imbricaria, Q. macrocarpa, Q. palustris, Q. prinus, and Q. velutina and field isolation from naturally infected Q. borealis, Q. ellipsoidalis, Q. macrocarpa and Q. velutina. Seven additional susceptible species of oak were reported in 1946 (12), Q. bicolor, Q. coccinea, Q. montana, Q. muehlenbergii, Q. rubra, Q. rubra var. pagodaefolia and Q. shumardii. Barrett (4) in 1947 listed a total of 24 species and varieties all susceptible to oak wilt. Of those Q. falcata, Q. garryana, Q. hemisphaerica, Q. laevis, Q. phellos, Q. shumardii var. texana and Q. suber had not previously been listed. Dietz and Young (14) further extended the knowledge of the host range to include four additional species: Q. gambelii, Q. laurifolia, Q. nigra and Q. virginiana var. maritima. Of 28 species and varieties tested by greenhouse inoculation all were susceptible to wilt.

The symptoms of oak wilt were described briefly by Henry and Moses (21) and more completely by Henry et al. (22). On red oaks the first symptoms of wilt were reported as slight crinkling and paling of leaves, usually near

the top of the tree or the tips of lateral branches. Affected leaves turned bronze and brown and the symptoms appeared progressively downward and inward on the tree, usually appearing last on the lower branches. Varying degrees of defoliation were noted and mature wilted leaves were observed to fall at all stages of symptom development. Young leaves became very dark as they wilted and did not show the bronzing typical of older leaves. Brown to black discoloration was observed frequently in the sapwood of diseased trees but was not always present. Small twigs were killed first, then the branches, trunk and roots. Red oaks always were killed once they were infected.

Progress of the disease was markedly different on trees of the white oak group. Comparatively few trees were found diseased and infection was less severe. Twigs with wilted, tan to brown leaves were often scattered throughout the tree. Some branches showed symptoms while others remained outwardly healthy throughout the season. A dieback of the branches was also observed on white and bur oaks.

Dietz and Young (14) reported the symptoms of oak wilt to vary with species of oak and with the season of the year in which infection occurs. When red oak trees were infected in late summer or early fall, leaf development was retarded in the following spring and only a few small leaves appeared scattered over the tree. These soon wilted and died. Symptoms on white and bur oaks were much more localized and trees often were observed to live for several years following infection with only a few branches dying each year. Such localized infection and killing commonly resulted in a stag-headed effect on white and bur oaks.

The oak wilt pathogen was described in 1944 (20) and placed in the

genus Chalara Corda of the Dematiaceae, to which four other species saprophytic on oak had previously been assigned. No perfect stage was found nor was the conidial stage observed in nature. However, the gray to olive green fungus was easily isolated from infected oak trees and chains of endogenous hyaline conidia were abundant in cultures two to three weeks old. The pathogen was isolated from roots, bole, branches, twigs and leaves of infected trees (23). Successful isolations from wound inoculated trees were made from branches bearing wilted leaves. In the bole the organism was confined to current growth and one and two year old wood.

The optimum growth temperature for Chalara quercina in culture was 24 - 28° C. on malt, corn meal and nutrient dextrose agars (20). Growth averaged 1.8 to 3.2 cm. in a week's time. Slight growth appeared after two weeks at 8° C. and 32° C. Similar growth was obtained on aseptic living and dead oak wood in the laboratory. Highest spore germination was in 1.25 per cent malt solution after 14 hours at 24° C. Germination was lowest in distilled water. Spores germinated by a single germ tube usually arising at one end of the spore or by two germ tubes, one at either end. If allowed to remain in water or malt the germinating spores gave rise to secondary endogenous spores.

Barrett (4) found that potato-dextrose, corn meal and nutrient agars supported slightly more rapid growth than malt, oatmeal and 2 per cent water agars. On 2 per cent agar growth was rapid but flat on the surface of the medium with only a few scattered tufts of aerial mycelium. Growth similar to that on potato-dextrose-agar was obtained on sterilized twigs of American elm, bur oak, red oak and shagbark hickory. Slow growth was observed on butternut and walnut. Excellent growth with abundant aerial hyphae was made

on red oak sawdust.

No satisfactory method of controlling oak wilt has been found. Apparently the use of resistant varieties offers no possibilities. Host specificity does not seem to be an important factor as limited tests showed that isolates from black, red, bur and scarlet oak were pathogenic on black oak and an isolate from black was pathogenic on white (23).

Dietz and Barrett (13) reported that sanitary methods checked and sometimes controlled the spread of oak wilt. Tests in which all infected oaks in an area and all healthy red oaks within a 50 foot perimeter were removed gave control of wilt (14). Results from plots in which only the dead and visibly diseased trees were removed were variable. In some plots spread was reduced, in others stopped. Pruning of infected branches was suggested as a means of saving white oaks with localized symptoms, although good results were not consistently obtained.

Mechanism of Wilt Induction by Vascular Parasites

Wilting associated with the vascular invasion of plants by fungi and bacteria has been attributed to mechanical interference with water movement through the vascular tissue and to the toxic action of metabolic products of the invading organisms. The toxin theory has found strong support in studies on several wilt diseases, particularly in recent years. Healthy excised plants developed typical symptoms when placed in extracts from cultures of the pathogens responsible for the Rangpur disease of tobacco (28), Fusarial wilts of cotton (39), tobacco (51) and tomato (9, 17, 37, 43), Cephalosporium wilt of daisies (15), Helminthosporium blight of oats (35)

and Dutch elm disease (54).

Numerous other theories have been advanced. One of the first suggestions was that of Atkinson (3) who demonstrated the vascular nature of the cotton wilt organism. Atkinson postulated that wilting was chiefly a nutritional reaction in which plants wilted because the fungus utilized nutrients present in the tracheal sap and starved the host. Others hypothesized (6, 19, 42) that wilting was due to plugging of host plant vessels by the accumulation of the pathogen and its by-products in sufficient quantity to obstruct the normal upward flow of water. In contrast there have been reports that the mycelium in vessels of wilting plants was sparse and grew closely along the inner vessel walls, (7). Occlusion by gums and tyloses has also been suggested but whether theirs is a primary or secondary role is not known definitely (24, 34). Wilting of potatoes, by species of Fusarium, was attributed to root necrosis which reduced the water absorbing power of the plant (30, 33). It was suggested that the wilting in flax caused by Fusarium lini might be due to the combined effect of root necrosis, increased transpiration, and toxic substances produced by the pathogen (45), or to the breaking of water columns in the host by gases produced by the causal organism (46).

In addition to the production of symptoms on excised cuttings other recent findings have greatly substantiated the toxin theory. Wellman (48) reported differences in the toxicity of culture filtrates of mild and virulent strains of Fusarium lycopersici. Gottlieb (17), with the same organism, demonstrated the presence of toxins within the xylem of wilted tomato plants. When oat varieties susceptible to Helminthosporium blight were placed in Helminthosporium culture filtrates, typical disease symptoms were

produced (35). Resistant oat varieties treated similarly were unaffected. Of more immediate import to the oak wilt problem was the demonstration of the production by Ceratostomella ulmi of a substance which produced typical Dutch elm disease symptoms when inoculated into healthy young elms (54).

The greatest amount of work in toxin production has been with vascular parasites in the genus Fusarium. Various compounds have been identified as the toxic agents in Fusarial wilt diseases. Among those compounds to which wilting has been attributed were an aldehyde (29), nitrites (39), amines (31, 41), ammonia (16), an alcohol (32), and a polypeptide like compound (37, 38). Hodgson et al. (25) were able to induce wilting in tomato cuttings with a glucosan obtained from cultures of Agrobacterium tumefaciens.

Numerous theories have been advanced to explain the action of these materials on the host plant. It was suggested that, in wilt diseases of tomato (5) and plum (24), toxic substances were secreted which stimulated living cells to produce gums that obstructed the vascular tissue. In Dutch elm disease (54) toxic action on host cells was considered the direct cause of wilting. Hursh (27) believed toxins in filtrates acted on the exposed vascular tissue of cuttings causing their collapse and interfering with water uptake. By cutting off the ends of stems and placing the cuttings in water the plants were revived.

Several reports have indicated that toxins may act on the leaves. Certain Fusarial toxins were thought to reduce the permeability of cell membranes (9). Thatcher (43) correlated an increase in permeability with the wilted condition. He believed that increased permeability promoted more rapid transpiration, resulting in progressive wilting which became permanent and led to the death of the mesophyll cells. Dowson (15) reproduced the

yellow leaf symptoms typical of Cephalosporium wilt of daisies by placing excised leaves in culture filtrate. More recently it was reported (26), that in tomato cuttings wilted by polyethylene glycols the greatest accumulation of glycols was found in the region of injury in the leaflets. Assuming that these materials moved through the plants on a diffusion gradient, these results would indicate some mechanism of fixation or precipitation of the toxic substance in the injured cells.

From the literature on the subject it appears that wilting may be due to a combination of several factors which act in one manner or another to upset the water balance in infected plants. However, the voluminous literature on the subject tends more and more to substantiate the toxin theory of wilting.

METHODS

Isolation and Culture of the Pathogen

The cultures of Chalara quercina H. used in these studies were isolated from naturally infected oak trees. The isolates were maintained on potato-dextrose-agar containing 20 g. of agar, 20 g. of dextrose and the extract from 200 g. of potatoes per liter.

Isolations from young twigs, petioles and leaves were made using the technique described by Barrett (4). Sections of twigs, two years old or younger, were surface sterilized in a 20 per cent concentration of commercial Clorox for one minute and rinsed in sterile distilled water. The twig sections were placed on a glass slide which had been dipped in alcohol and flamed, and serial cross sections were cut with a sterile razor blade. Seven sections were placed on edge in the medium in each petri dish and the dishes were incubated at 20° - 25° C. All cultures were identified microscopically and mass spore and mycelium transfers made to potato-dextrose-agar slants.

Different techniques were used for the isolation of Chalara from older branches. In isolating from branches 1 to 4 inches in diameter alcohol was poured over the bark and flamed off. Cross sections were cut from the region of the cambium with a sterile razor blade or scalpel and placed on edge in potato-dextrose-agar. Isolations were made from larger branches, on which the bark was rough and cracked, by breaking off the bark with a wood chisel and taking sections which included one and two year old wood.

A special method of isolation was devised for studying the distribution of the fungus within the bole of the tree. Complete cross-sections, one inch thick, were taken at consecutive 8 to 12 foot intervals up the bole. A radial strip 2 inches wide and extending the complete diameter of the cross-section was taken from each disk. With a circular saw these strips were sawed half way through at the cambium, between the second and third growth rings and at one inch intervals across the remaining distance. In the laboratory the partially sawed sections of wood were easily broken off by inserting a wood chisel in the saw path and exerting lateral pressure. A portion of wood was cut from each exposed surface and placed on potato-dextrose-agar.

Study of Diseased Tissue

Diseased tissues were studied by histological methods. Sections of diseased leaves, petioles and young stems were killed in Craf III or FAA and dehydrated with normal butyl alcohol (40). The tissues were infiltrated with paraffin, cast in Parlax and sectioned with a rotary microtome. Older stems were killed in FAA and sectioned with a sliding microtome.

Satisfactory staining resulted when the sections were mordanted in an aqueous solution of 1 per cent potassium metabisulfite and 1 per cent tannic acid for 30 minutes, stained for 30 minutes in hemalum and counter-stained for one hour in safranin. When stained in this manner mycelial walls were dark red and protoplasmic inclusions were dark red to purple.

Spore Germination

Spore germination tests were conducted in connection with the physiological and chemical inhibition studies. Spores were germinated in petri dishes on 2 per cent agar containing 1 per cent dextrose. Conidia from 10 day old test tube cultures of Chalara were used for all tests. The cultures were incubated at 20° C. to facilitate abundant sporulation and lessen the possibility of accumulation of metabolic products inhibitory to germination. The spores were not washed since unwashed checks consistently gave 95 to 100 per cent germination. Spore suspensions were made by the addition of sterile distilled water to the test tube cultures followed by the necessary dilutions to give 50,000 spores per ml. of liquid. Four .1 ml. droplets of conidial suspension were equidistantly pipetted onto each petri plate. Germination counts were taken at various intervals from 16 to 96 hours depending on the nature of the tests.

Germination tests were also attempted in liquid droplets of various nutrients. Such counts were unsuitable for three reasons. (1) Endospores were rapidly produced from germ tubes of germinating spores and in liquid floated away from the conidiophores, confusing the count. (2) Spores within the liquid did not germinate as well as those at the surface of the liquid. (3) It was impossible to observe the germination-sporulation cycle discussed under results.

Inoculation

Virulence of an isolate from one species of oak was determined for

other species by greenhouse cross-inoculation studies. Three methods of inoculation proved equally effective.

1. A longitudinal slit was made in the bark of the young stem and a weft of mycelium, spores and agar inserted. The wound was wrapped with moist cheesecloth.

2. A conidial suspension was sprayed over the young plants. With a sterile scalpel or needle, longitudinal slits were made sufficiently deep to introduce spores into the xylem.

3. Conidial suspensions were hypodermically injected into the outer xylem.

Inoculated plants were kept in a moist chamber for 48 hours after which time they were transferred to a greenhouse bench. Symptoms typically appeared on apical leaves after 10 to 20 days. No successful inoculations were obtained without wounding the host.

Field inoculations were made by removing a small (3/4 to 1 inch) rectangle of bark and filling the opening with a mass of agar, mycelium and spores. The opening was then covered with moist cheesecloth.

EPIPHYTOLOGY

Information on the method of overwintering and natural spread of the pathogen is essential to the development of a disease control program. Detailed observations were made on field plots established in Dolliver, Call and Pikes Peak State Parks and in state forest areas near McGregor.

Method of Overwintering

According to present knowledge, the fungus overwinters only in infected trees. White and bur oaks are the most common reservoirs of infection. Persistence of the pathogen in these trees for several years has been shown by isolation from infected trees over a period of three years.

Overwintering is less common on red oaks since they are killed quickly, but may occur when trees are infected in late summer or fall. Red oaks so infected usually die early in the following growing season. The fungus may be isolated readily from roots, bole, branches, petioles and leaves of such trees.

The pathogen has been observed to persist in stumps for two years after infected red and white oaks were removed. Sprouts which grew from such stumps usually developed typical wilt symptoms by midsummer.

Pattern of Spread

Spread of the oak wilt fungus from diseased to healthy trees may be assumed to occur in one or more of three different ways: (1) by wind blown

spores, (2) by an insect vector, or (3) by other methods that would serve to transfer fungus particles from diseased to healthy trees. Root anastomosis, bark feeding rodents such as deer mice or squirrels, or mechanical wounding might function in disease transfer.

In order to obtain information on field spread, plots were established in isolated areas where one or only a few trees were infected. Plots were laid out with a rod and transit and maps were made of the location of all trees in each area. Records were taken periodically upon the amount of infection as determined by observation of symptoms and isolation of the pathogen. Data from 4 typical sites of infection are shown in Figure 1.

In McGregor Plots 1 and 2 the disease spread to the nearest adjacent trees on one side of the point of initial infection. Not all trees within the range of spread were involved. The greatest distance of spread within a single year was 45 feet. In McGregor Plot 3 and Delliver Plot 34 the disease spread concentrically to the nearest trees. No infection was recorded beyond those trees immediately surrounding the first point of infection. The average distance of spread was about 30 feet.

These data do not coincide with the typical pattern of spread by air borne spores (49) or insects (52, 55). If infection were by either of those agencies it would be expected that the incidence of infection would vary inversely with the logarithm of the distance (55) and spread would be expected over a greater area than was observed.

Maps of infected trees (Figure 1) show that such spread is not usual. In general the disease was not spread over large distances but to the nearest surrounding trees. In evenly distributed oak stands spread occurred in a roughly concentric pattern from the locus of infection outward. In areas

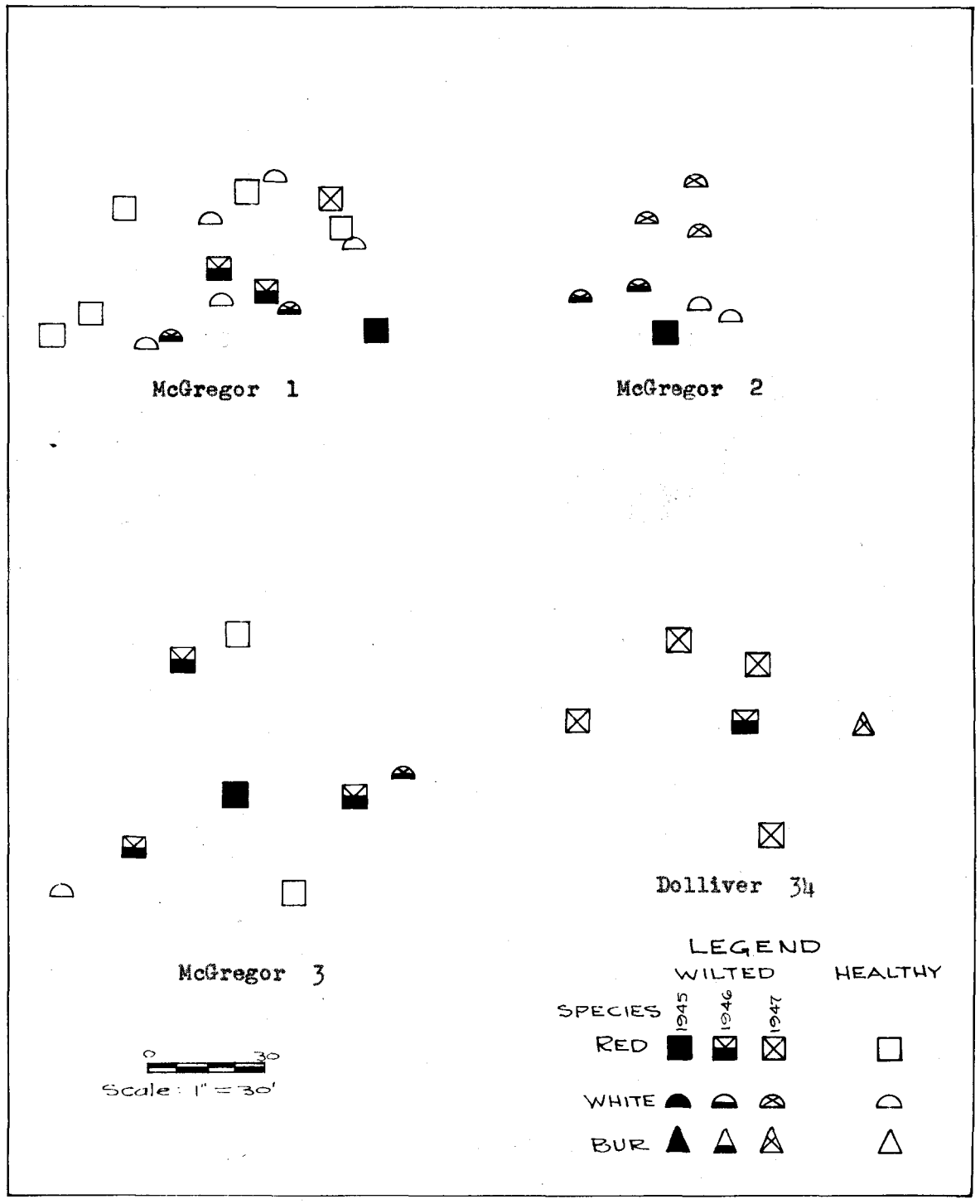


Fig. 1. Distribution of wilted trees in four different localities within two years after initial infection.

where the disease had been active for several years it was possible to observe the same slow outward pattern of spread by the relative deterioration of dead oaks.

Size of Trees Most Commonly Affected

In many diseases susceptibility to attack by the causal organism varies greatly with age and maturity of the host. Oak wilt is most obvious on large trees of the red oak group and it has been suggested that the large dominant trees in an area are the first attacked (12). To test for a correlation between size of tree and infection complete surveys were made of all the oak trees in 8 wilt-infested areas. Species and diameter-at-breast-height (DBH) were recorded. Typical data on the distribution of infection on red and white oaks in two of these localities are given in Figures 2 and 3. In general the number of infected trees of a given size was proportional to the total number of trees of that size in an area. However, the mean DBH of the infected white oaks was slightly lower than the mean for the total white oak population. It may be noted that the mean DBH for trees in the northeastern part of the state (Figure 3) are from 3 to 6 inches greater than for trees in the north central part of the state (Figure 2). This observation was true for other plots and species in the same general regions.

There is apparently no difference in susceptibility of trees of different sizes to oak wilt since trees of all sizes were killed in proportionally equal numbers.

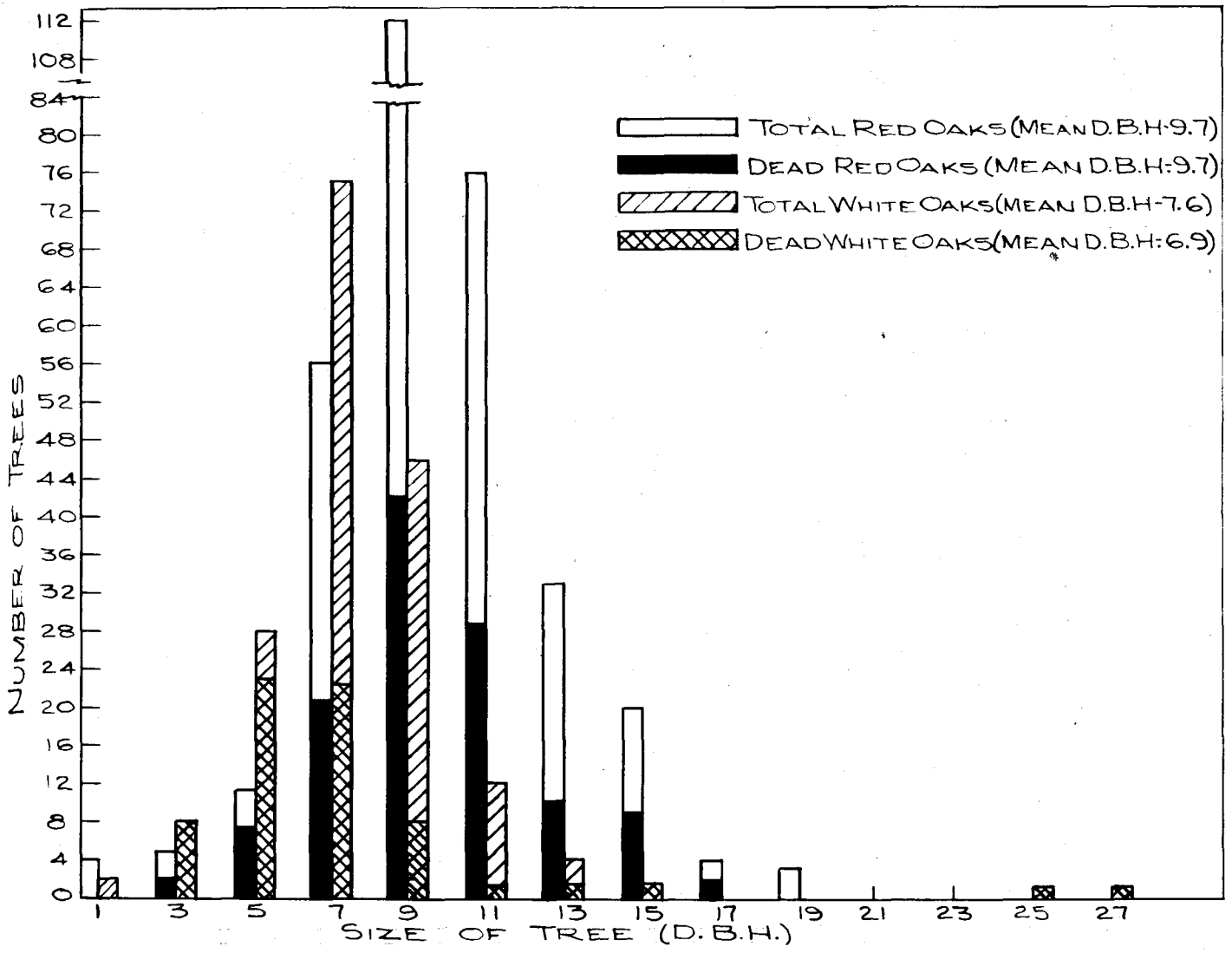


Fig. 2. Total number of red and white oaks of different sizes at Plot 1, Dolliver State Park and number of dead oaks in each class.

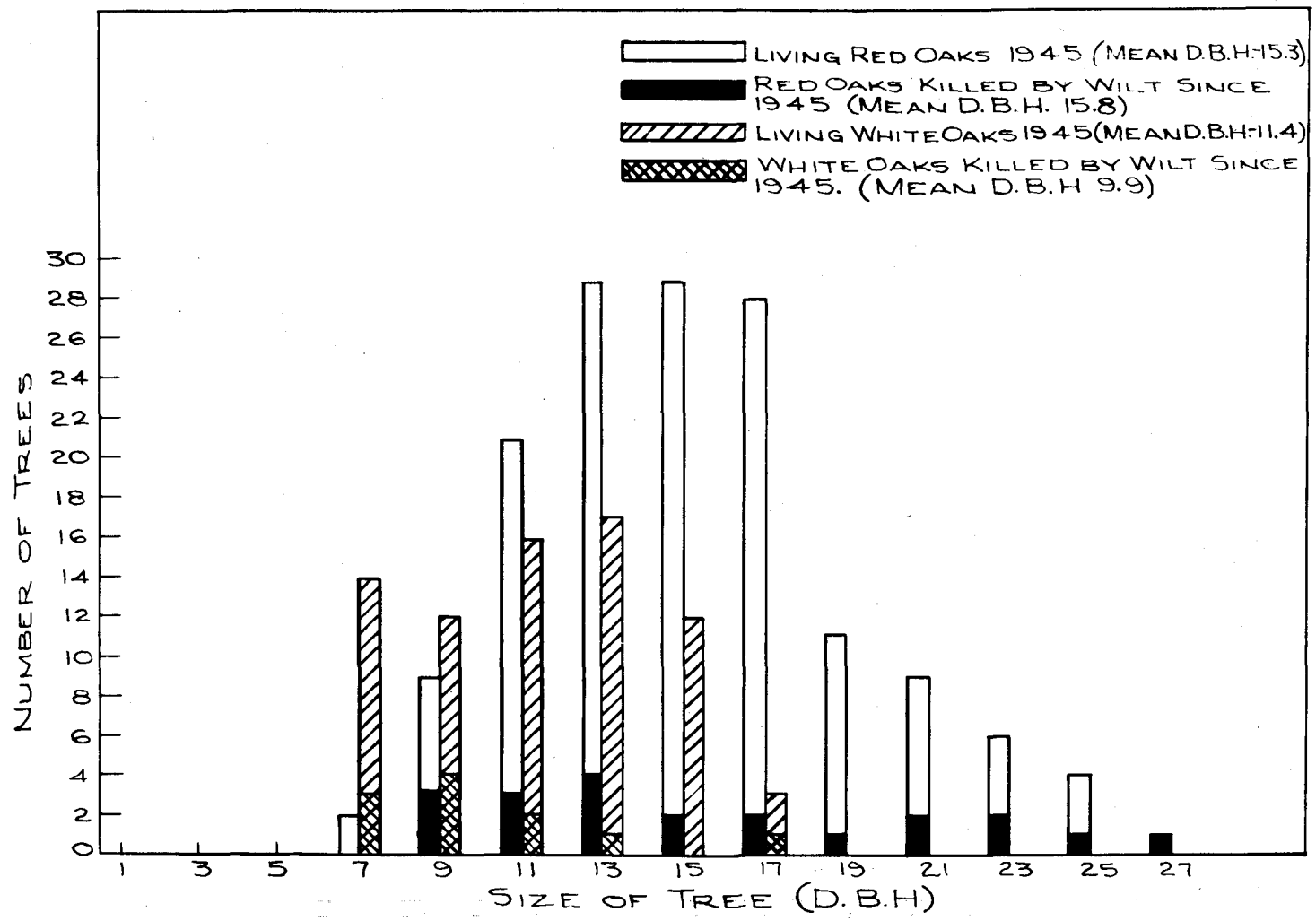


Fig. 3. Number of living red and white oaks of different sizes Plot 1, Point Ann, McGregor, in 1945, and number in each class killed since 1945.

Percentage Infection of Different Oak Species

It has been a general observation that oak wilt is more destructive on trees of the red oak group than on those of the white oak group (12, 22, 23). The above observation holds true from the standpoint of speed of destruction of both naturally infected trees in the field and artificially inoculated greenhouse oaks. Regardless of the source of inoculum, trees of the white oak group may survive indefinitely after infection whereas those of the red oak group are usually killed quickly.

To compare the incidence of natural infection of red and white oaks, data were tabulated on the percentage of the total volume of red and white oaks infected in 1946-47 in areas at Dolliver State Park and near McGregor.

Table 1. Percentage of natural infection of red oak (Q. borealis) and white oak (Q. alba) at four localities in northern Iowa in 1947.

Location of plots	Red oak			White oak		
	Total	Wilt incidence		Total	Wilt incidence	
	no.	no.	%	no.	no.	%
Marquette Pt. I	60	6	10	89	6	6.7
Marquette Pt. III	39	7	17.9	41	1	02.4*
Point Ann I	149	21	14.0	74	11	14.8
Dolliver I	90	10	11.1	50	6	12.0
Totals	338	44	13.0	254	24	9.4

The data in Table 1 indicate that there is little difference in the susceptibility of red and white oaks to infection by Chalara quercina H. The extremely low figure for infection of white oaks in Marquette Point III is probably due to the unequal distribution of the species near the locus

of infection in that plot. In the other plots red and white oak stands were better mixed.

At Pilot Knob State Park in north central Iowa, dead and infected trees were so generally distributed throughout the park as to preclude study of spread from a single locus of infection. However, several areas were cruised and the number of dead and healthy trees of each oak species recorded. The results are shown in Table 2.

Table 2. Relative destruction of different species of oak at Pilot Knob State Park.

Species	Living	Dead	Total	% dead
Bur (Q. macrocarpa)	1628	405	2033	19.9
White (Q. alba)	215	85	300	28.3
Hill's yellow (Q. ellipsoidalis)	752	936	1688	55.4
Red (Q. borealis)	411	463	874	52.9
Totals	3006	1889	4895	38.6

The percentages of the Hill's yellow (55.4) and red oak (52.9) trees that were dead in the areas cruised were approximately twice as great as in white oaks (28.3) and almost three times as great as in bur oaks (19.9). However, large areas of dead bur oaks were found and numerous trees with a few dead branches were observed.

These data bear out the observation that oak wilt is most destructive on species of the red oak group.

Host Specificity

Because of the wide variation in type of symptoms on different species

of oak and the variation in rate of development in white oaks tests were made for host specificity of different isolates. Single spore cultures of isolates from six different species of Quercus were inoculated into healthy young greenhouse trees of seven different species.

As shown in Table 3 each isolate was pathogenic on all oak species tested. Time required for symptom production and type of symptoms produced by each isolate were comparable. In addition to the species listed in Table 3 numerous others were tested in the course of host range studies. No host specificity was observed in the different isolates.

Table 3. Reaction of seven species of oaks to *Chalara quercina* isolates from six different sources.

Species inoculated	Infection produced by culture from					
	Red	White	Bur	Hill's yellow	Black	Black jack
Red	+	+	+	+	+	+
Hill's yellow	+	+	+	+	+	+
Pin	+	+	+	+	+	+
Bur	+	+	+	+	+	+
White	+	+	+	+	+	+
Black jack	+	+	0	+	+	+
English	+	+	0	+	+	+

+ = inoculation positive

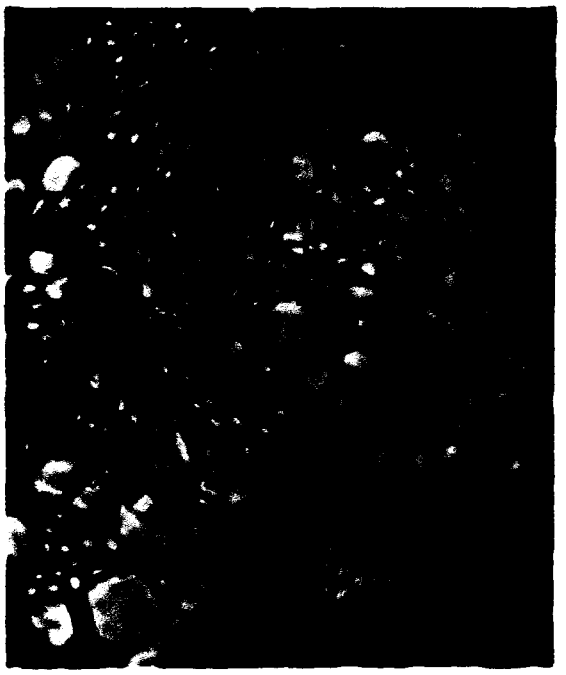
0 = not tested

DISTRIBUTION OF THE FUNGUS IN DISEASED TREES

The sudden and complete wilting of large oak trees suggests that Chalara quercina is a typical vascular parasite. If so, wilting would be induced by mechanical plugging of the vascular system, by production of a toxic substance which diffuses throughout the tree or by rapid growth or transport of the pathogen from the site of infection throughout the tree. The distribution of the pathogen in 5 naturally infected red oaks was determined by host tissue isolations from all parts of the tree.

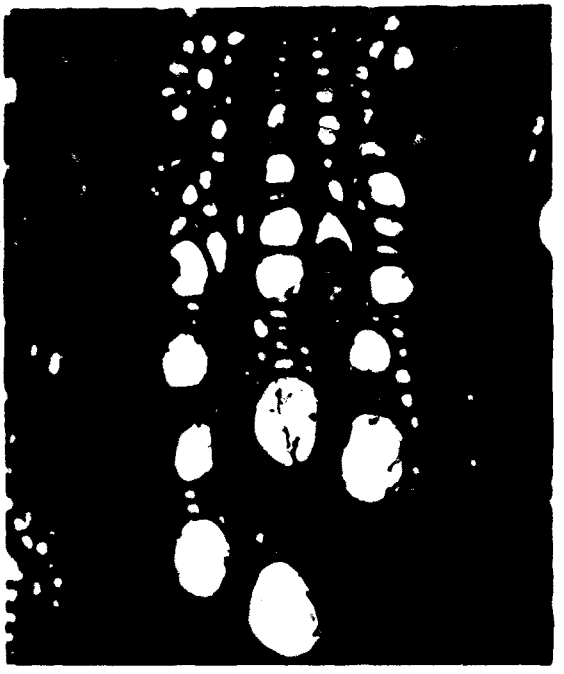
The pathogen was isolated from leaf midribs and petioles of wilting leaves, from branches and twigs bearing wilting leaves and from the bole. Isolations from twigs on lower branches bearing apparently healthy leaves did not yield the organism. The fungus was found throughout the bole perpendicularly but was not always found around the entire circumference. Radial distribution was restricted to the outer 1/4 inch of sapwood. The fungus was never isolated from the heartwood.

Results of the tree analyses by isolation were verified by study of microtome sections of diseased tissue from 5 red oaks and 3 white oaks. In these sections mycelium was observed in xylem vessels of leaf midribs, petioles and twigs (Figure 4). Mycelium was apparently confined to the xylem and was not present in all vessels. Most of the hyphae grew longitudinally in the vessels (Figure 5 A-B). Mycelial development was sparse in general but in a few vessels many hyphae were observed (Figure 5 C). Numerous conidia were observed in the vessels (Figure 5 D). These could be distinguished readily from excised hyphal tips and sections by the bipolar

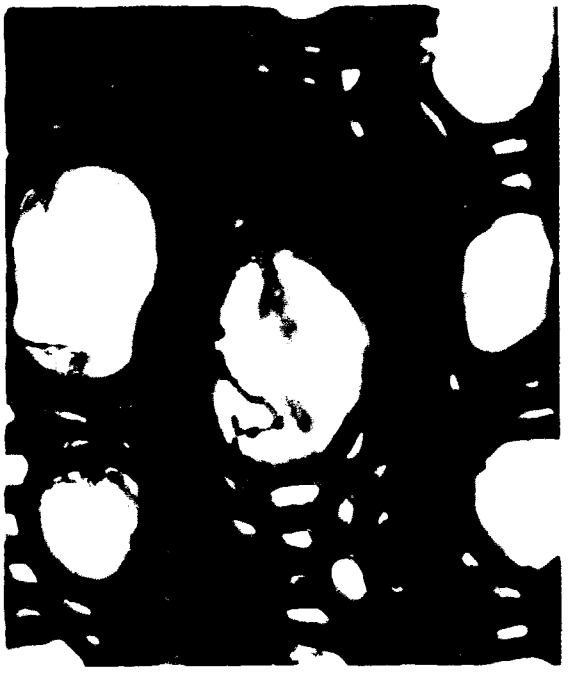


A. Petiole 225x

B. Petiole 475x



C. Stem 225x



D. Stem 475x

Fig. 4. Cross-sections of red oak petiole and twig showing mycelium in vessels.

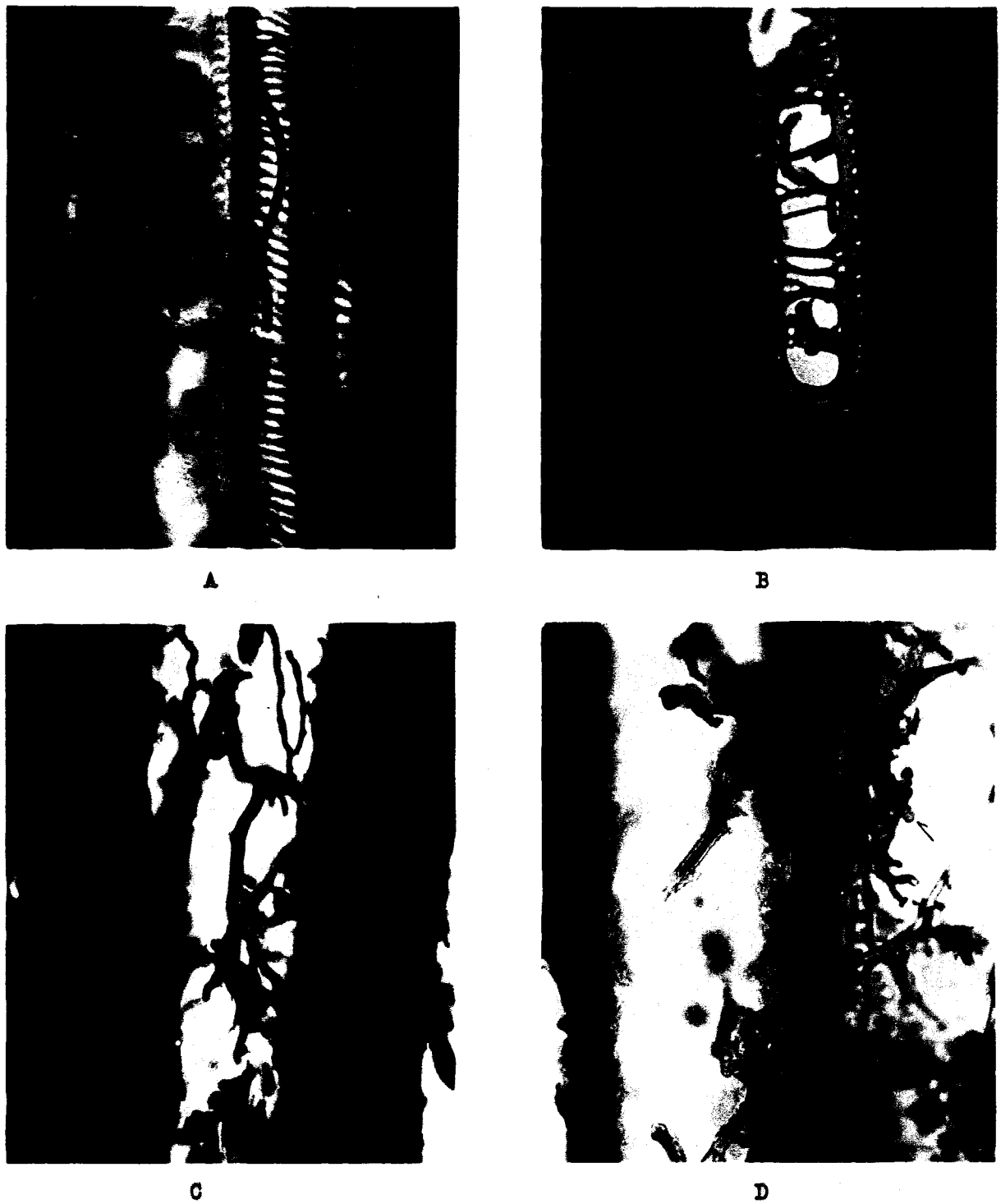


Fig. 5. Longitudinal sections red oak twig. 475x. A,B,C - mycelium in vessels. D, mycelium and spores (arrows) in vessels.

droplets which were present in the conidia.

These analyses show that the pathogen was usually present in wilted leaves and in twigs and stems bearing wilted leaves. Thus wilting is not necessarily due to a toxin transported from other locations in the tree nor to mechanical plugging. The presence of the small (2 to 3 x 5 to 7 μ) conidia in vessels would provide a mechanism for rapid dissemination of the fungus throughout the tree in the transpiration stream. Vessels of both red and white oak, even those in leaf veins, are large enough to facilitate passage of the spores.

To test the movement of Chalara conidia through stem sections, spore suspensions were drawn by suction through 10 to 12 inch sections of living twigs. Conidia passed through the twigs in the first droplets of suspension, some in chains of 5 to 10 spores.

Vessels of white oaks were smaller than those of red oaks and more occluded with gums and tyloses. These anatomical features may in part account for the slower development of the disease in white oaks.

CULTURAL VARIATION OF DIFFERENT ISOLATES

Variations in cultural growth characteristics of different isolates were observed. Transfers of a culture when grown at different temperatures or on different media also showed a wide range of growth characteristics.

A group of 20-day-old Chalara cultures on potato-dextrose-agar slants are shown in Figure 6. Cultures range from heavy sporulating types with sparse mycelial development (A and B), through intermediate types with heavy mycelial development and abundant spore production (C and D) to cultures which produce abundant mycelium and very few spores (E and F).

Most isolates from naturally infected trees were of the C-D growth type with greenish surface mycelium and tufts or mats of grayish tan aerial hyphae and spores. Clear to amber liquid droplets commonly collected on the surface of 7 to 10 day old cultures. The heavy sporulating "B" type cultures were isolated very rarely as were "E" type cultures. "A" type growth occasionally developed from mass transfers from "C" type growth and on further transfer made typical C-D growth. Culture "F" was transferred from a sector on a C-D type colony and through several transfers did not revert back to the original type of growth. All of 15 cultures of C-D type growth except the one from which "F" was transferred have grown true to type over a two year period.

The differences in growth type exhibited might well be due to the dual phenomenon reported for numerous imperfect fungi by Hansen (18). By successive series of single spore isolations Hansen was able to isolate three culture types: (1) a mycelial type which produced few or no conidia, (2) a

Fig. 6. Twenty day old cultures of Chalara quercina on potato-dextrose-agar. A-B, heavy sporulating types. C-D, producing both conidia and mycelium abundantly. E-F, abundant mycelial development, few conidia.

conidial type which produced conidia in great abundance and usually less aerial mycelium, and (3) a type intermediate in conidial production and mycelial development.

Culture "B" would be comparable to Hansen's conidial homotype, "C" and "D" to the intermediate type and "E" to the mycelial type. Single spore isolations of Chalara quercina H. made in connection with other work on the problem failed to reveal any segregation of mycelial or conidial homotypes from the "C" and "D" type cultures. However, sufficient series of single spore isolations were not made to be comparable to Hansen's work.

EFFECT OF CULTURAL CONDITIONS ON DEVELOPMENT OF THE PATHOGEN

In order to obtain a better understanding of the effect of certain environmental conditions on growth and development of C. quercina, its cultural response to certain external stimuli was observed in the laboratory.

Effect of Temperature on Growth and Spore Germination

Daily growth increments of Chalara quercina on petri dishes of potato-dextrose-agar at 5, 10, 15, 20, 25, 30 and 35° C. were recorded. Plates were centrally inoculated with a .05 ml. droplet of spore suspension containing 250,000 conidia per ml. and were incubated for 48 hours at 25° C. to allow equal germination in all cultures. Plates were then placed in incubators at the various temperatures to be tested. Growth was recorded at 24 hour intervals by measuring the diameter in two planes at right angles and recording the average of the two. All tests were run in quadruplicate and repeated.

Most rapid growth occurred at 20° to 28° C. (Figure 7). No growth occurred at 35° C., and at 5° C. none occurred until after 10 to 14 days. Slow growth was recorded at 2° C. and 32° C. Growth at 10, 15 and 20° C. was linear with time. However, at 25° C. and 30° C. curves showed a slowing of growth after the maximum rate was reached. This inhibition was probably due to the accumulation of staling products of the fungus. Similar observations were made on growth in liquid culture. Cultures in yeast extract solution were autolysed after 90 days at 25° C. Cultures of the same age maintained at 20° C. were still vigorous.

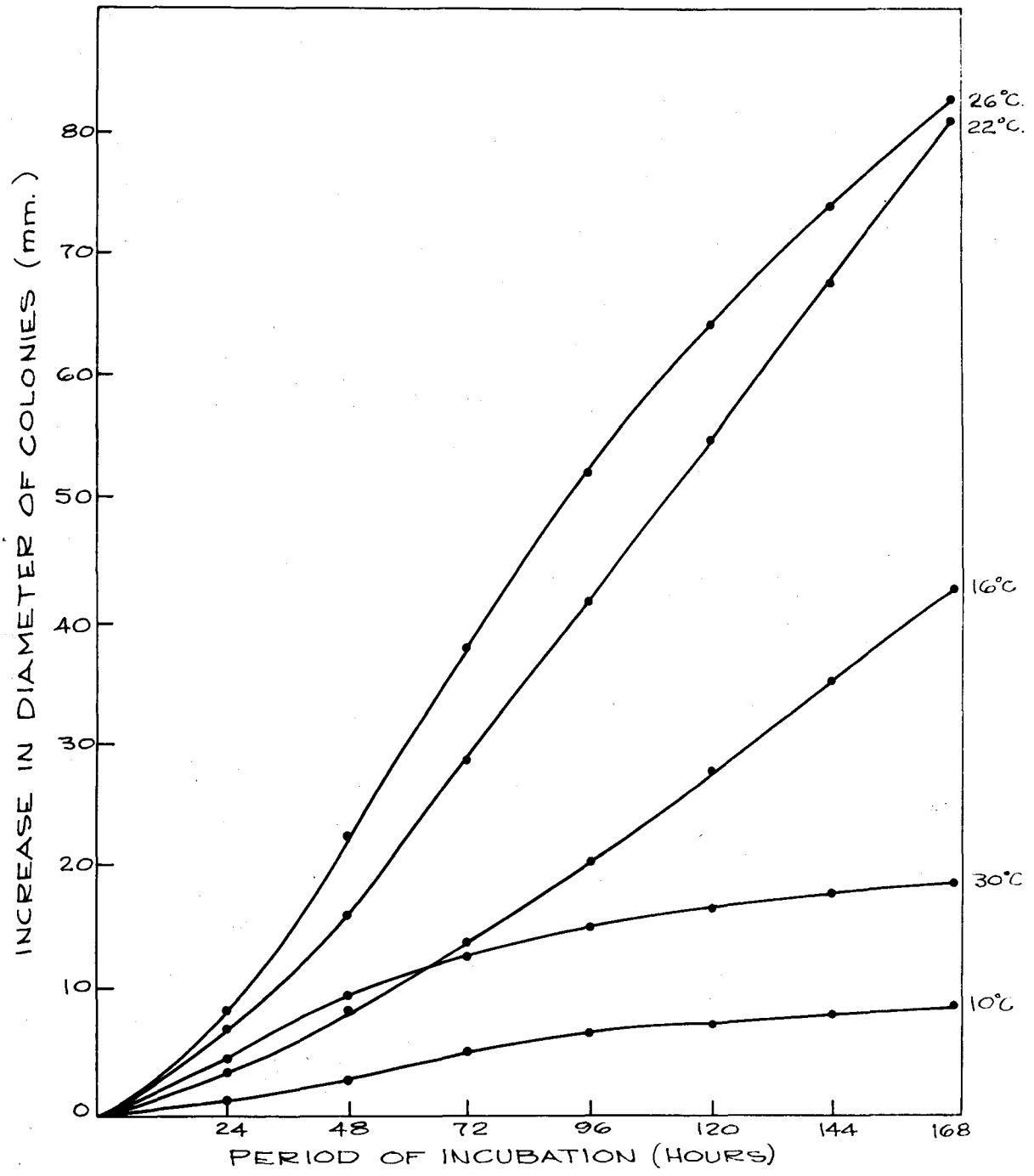


Fig. 7. Effect of temperature on growth of C. quercina.

Colonies grown at the various temperatures differed markedly in appearance. At 10° C. light-tan, crusty mycelial masses developed at the center of the colony of white surface hyphae. Growth at 15° C. was similar to that at 10° C. with more extensive development of light-tan aerial hyphae. Growth at 20° C. radiated evenly with dusty tan aerial hyphae and slight greenish pigmentation of the surface hyphae. At 25° C. surface hyphae radiated evenly but aerial mycelial development was irregular with masses of aerial hyphae in patches and concentric rings. Dark green pigmentation was pronounced in both surface mycelium and the agar. Mycelial development was more abundant than at the lower temperatures. Mycelial growth at 30° C. was compact and heavily pigmented and development of tan aerial hyphae was sparse.

Spore germination tests were conducted over the same temperature range. Maximum spore germination occurred at 25° to 30° C. (Figure 8). At this temperature 92 to 95 per cent of the spores germinated within 16 hours and up to 100 per cent after 24 hours. Germination was slow at the lower temperatures. At 10° C. 2 to 5 per cent germination was recorded after 96 hours. No germination occurred at 35° C. and spores which germinated at 32° C. did not continue to develop.

The method of germination differed markedly within the optimum temperature range. After 48 hours at 25° C. from 95 to 100 per cent of the germinating spores had produced numerous endospores (Figure 9) the oldest of which had also germinated and were producing a second group of endospores (Figure 10). At 30° C. the majority of the germinating spores developed into vegetative hyphae and less than 15 per cent were producing endospores after 48 hours. Endospores were produced abundantly after 48

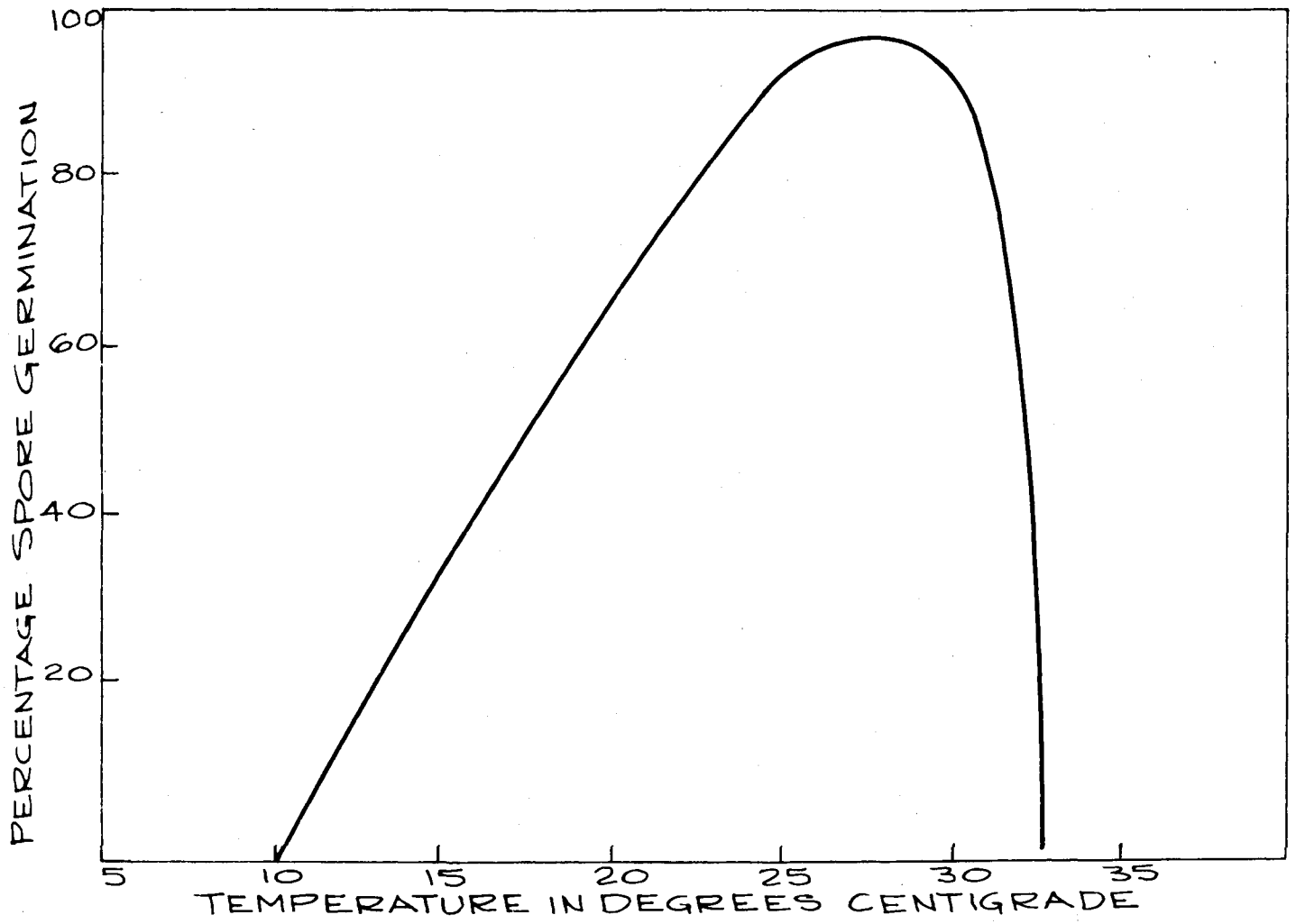


Fig. 8. Percentage of spore germination after 24 hours at various temperatures.

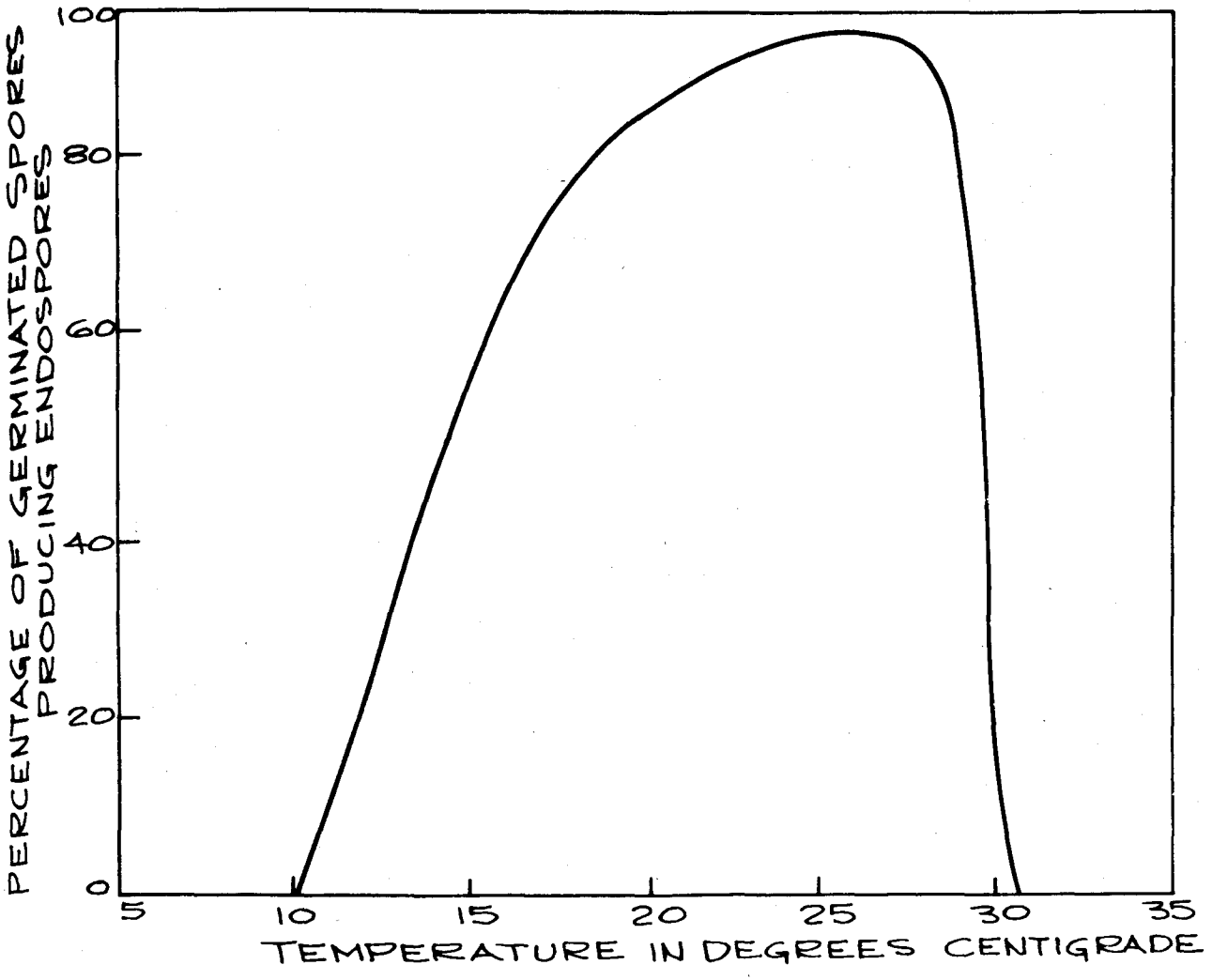
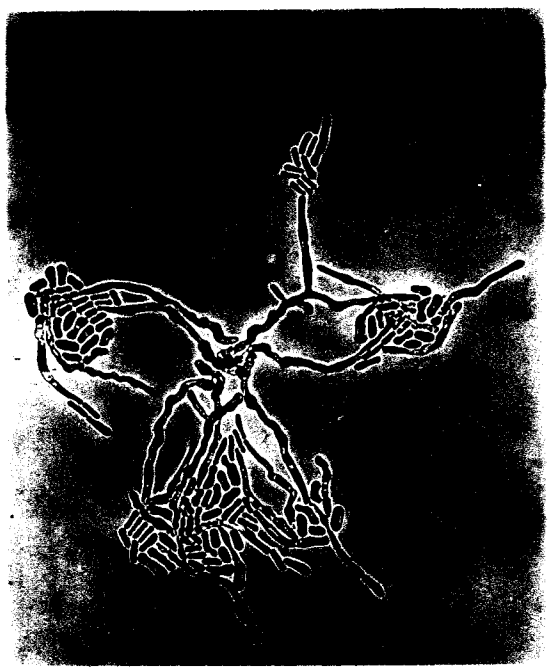


Fig. 9. Effect of temperature on production of endospores from germ tubes of germinating spores after 48 hours incubation.

15° C.

20° C.



25° C.

30° C.

Fig. 10. Production of endospores after 48 hours at 15°, 20°, 25°, and 30° C. (300x)

hours at 20° C. and more slowly at 10° and 15° C. After 96 hours spores had accumulated in large masses at 20° C. and 25° C. (Figure 11). All spores that germinated at 15° C. were producing endospores but at 30° C. endospore production was very limited.

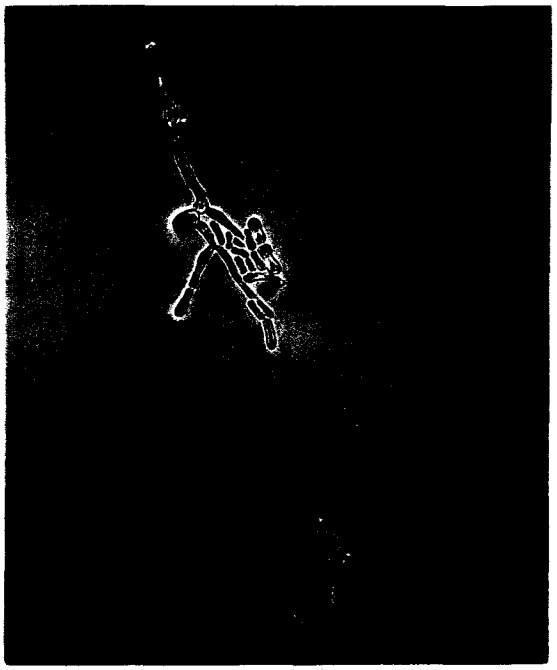
Rate and Type of Growth on Different Nutrients

The rate of growth on several media was determined. All media contained 2 per cent agar, 2 per cent dextrose and the additional nutrients shown in column 1, Table 4. Plates were inoculated as in the temperature studies then incubated at 25° C. Measurements were taken at twenty-four hour intervals for a period of nine days after which time those media on which most rapid growth was made were covered. Column 3 shows the daily rate of increase of diameter, in millimeters, at the peak of growth on each medium. The measurements represent an average of 4 replications.

Table 4. Relative effectiveness of different nutrient substrates in supporting mycelial growth of C. quercina.

Supplementary nutrient	Conc. g./l.	Mean daily growth increments
		mm.
Casein	15	6.0
Yeast extract	5	7.3
Potato	200*	12.0
Malt	15	6.5
Oat meal	50	11.0
Asparagine	5	11.5
Asparagine + $ZnSO_4$	5 + .005	11.0
Check	—	7.0

* Extract from 200 grams of fresh potatoes.



1. 15° C.

2. 20° C.

3. 25° C.

4. 30° C.

Fig. 11. Production of endospores after 96 hours at 15°, 20°, 25°, and 30° C. (1) 300x, (2), (3) and (4) 200x.

Nine days after inoculation, the potato, oatmeal and asparagine media were completely covered and further measurements were impossible. Mycelial development was abundant on the media containing a complete protein source and certain growth factors (casein, yeast extract, potatoes, malt and oats), and olive green pigmentation was pronounced. On the other media growth was thin, adhered closely to the surface of the medium and showed slight pigmentation. Mycelium, on the asparagine medium to which $ZnSO_4$ was added, developed a slight greenish pigmentation but not comparable to that on the complete nutrient media.

Spore germination counts were made on plates of 2 per cent agar containing various concentrations of dextrose. Better than 90 per cent germination was recorded over a concentration range of 0.5 per cent to 2 per cent (Figure 12). Germination was consistently above 80 per cent with as little as 0.1 per cent sugar in the medium, but dropped off sharply when no sugar was available. Better than 60 per cent germination was obtained at a dextrose concentration of 40 g. per liter. No higher concentrations were tested.

In addition to inhibiting spore germination, high concentrations of sugar were also unfavorable for the production of endogenous conidia by the germinating spores. The percentage of germinating spores that produced conidia from their germ tubes was lowest at the 4.0 per cent sugar level and increased as the sugar concentration decreased down to 0.1 per cent. On the medium containing no sugar, sporulation was at a slightly lower level than on the 0.1 per cent medium.

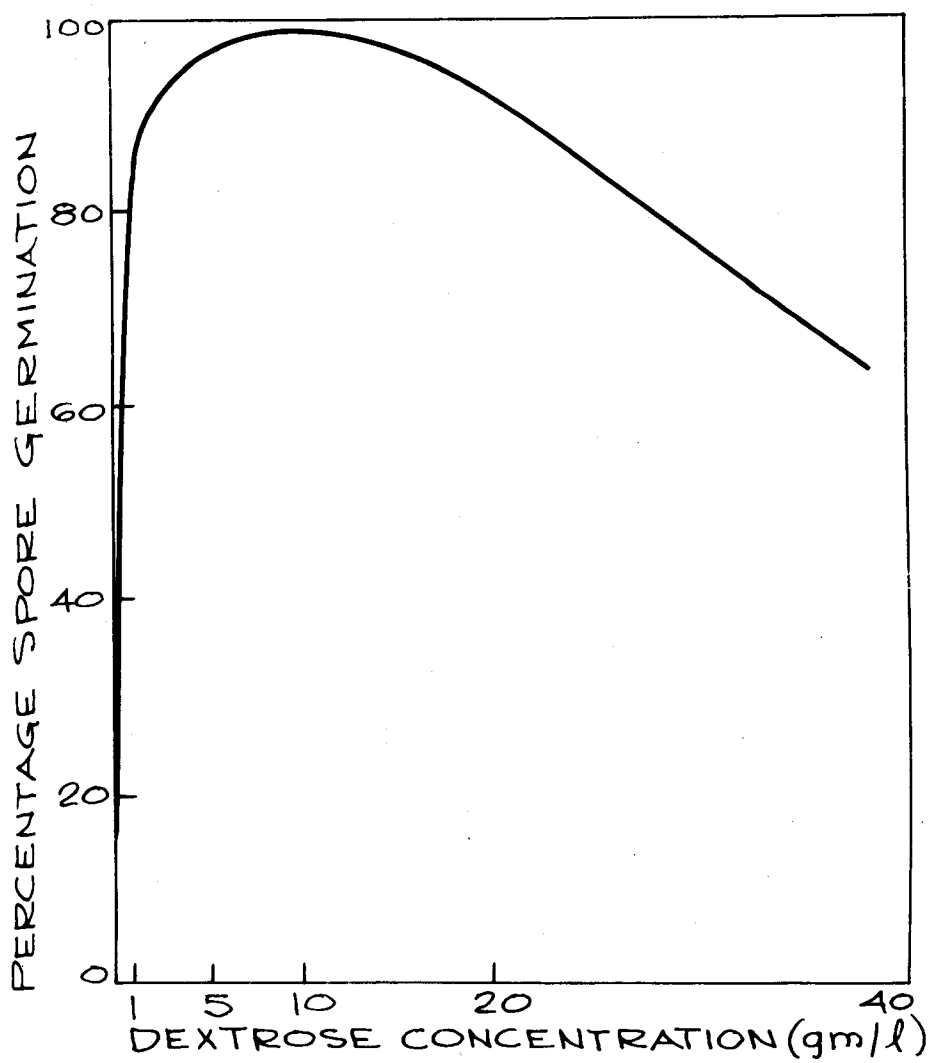


Fig. 12. Effect of dextrose concentration in agar medium on germination of spores.

Effect of Light on Growth and Spore Germination

The effect of light on spore germination, growth and sporulation of Chalara quercina was tested. Inoculated petri plates were placed in a two pound coffee can from which light was eliminated by lining with black paper. Plates in the open were exposed to diffuse daylight. To observe the effect of light on growth and sporulation plates were incubated for 9 days at 24° C., then the diameters were recorded. In testing the effect on spore germination and sporulation plates were removed from the can and counts taken directly.

Light apparently had no inhibitory or stimulatory effect on the processes observed. No differences were observed in growth type or pigmentation. Sporulation was abundant in both light and darkness.

Hydrogen Ion Tolerance

Tests were made to determine the optimum pH range for growth of Chalara quercina H. in culture. The medium used was a modified Richard's solution to which asparagine and yeast extract were added. The medium contained:

KNO_3	5 g.	Dextrose	25 g.
KH_2PO_4	2.5 g.	Asparagine	2 g.
$MgSO_4$	4.0 g.	Yeast extract	4 g.
$FeCl_3$	0.1 g.	Dist. HoH	1000 ml.

The unadjusted pH of this medium ranged from 4.8 to 5.3 and remained constant even after 60 days of active growth of Chalara. Adjustments to pH levels from 2 through 10 were made by the aseptic addition of the necessary quantities of 1/20 normal HCl and NaOH to sterilized and cooled 100 ml. por-

tions of medium in 250 ml. Erlenmeyer flasks. Four replicates were set up at each pH. Each flask was inoculated with 250,000 conidia and the flasks were incubated at 24° C.

All pH determinations were made with a Leeds and Northrup glass electrode pH meter. Acid and alkaline curves were established on test flasks of medium and additions made to the experimental medium accordingly. The amount of growth was recorded in milligrams of dry weight. The net weight in milligrams shown in Figure 13 represents the average dry weight of the mycelial residue at the various levels minus the dry weight of the residue from the pH 2 samples in which no growth was made.

Growth occurred over a range from pH 3.0 to pH 9.0 with the optimum from 5.0 to 7.0 (Figure 13). The greatest amount of growth was recorded at 6.0. No growth occurred at pH 2 or pH 10. Addition of sufficient NaOH to raise the pH level to 10 caused crystal formation in the medium and precluded taking of weights at that pH.

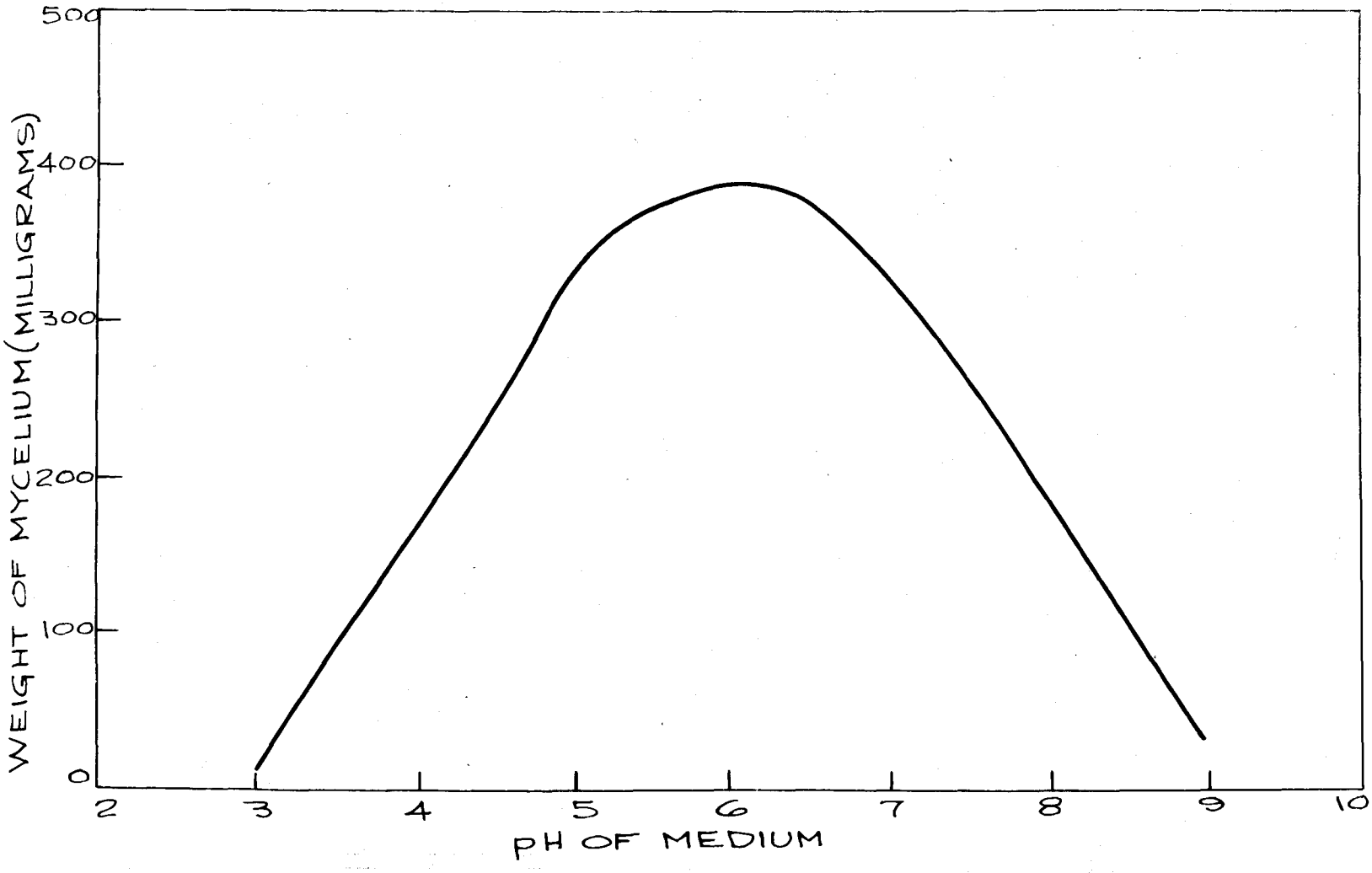


Fig. 13. Effect of hydrogen-ion concentration on growth of C. quercina.

INHIBITION OF GROWTH AND SPORE GERMINATION BY CHEMICALS

Since Chalara quercina grows and sporulates inside the oak tree there is need for a potent fungicide to serve as a chemotherapeutic agent. The effect of a number of chemicals in the inhibition of spore germination was tested in pure culture on 1 per cent dextrose agar. The agar, dextrose and water were autoclaved in 50 ml. lots, cooled to 45° C. and sufficient chemical added aseptically to obtain concentrations of 1000, 100, 10 and 1 parts per million of substrate. The medium was then distributed equally among 3 petri plates. After the medium had solidified four 0.1 ml. aliquots of spore suspension were equidistantly pipetted onto each plate. The cultures were incubated at 24° C. for 96 hours and records on germination and growth were taken at 48 and 96 hours. The number of spores germinated was determined by counting 35 spores in each of three locations on each plate. This method of spore germination was used in preference to the standard procedures recommended by the American Phytopathological Society's committee on standardization of fungicidal tests (1, 2) because it permitted observations on growth and sporulation as well as on spore germination.

The chemicals tested and the percentage inhibition of spore germination at various concentrations are shown in Table 5. Malachite green, crystal violet, 8-hydroxy quinoline (Bioquin), copper 8-hydroxy quinoline (Bioquin 1), 8-hydroxy quinoline benzoate (Bioquin 700), 8-hydroxy quinoline sulfate (Bioquin 850), and phenyl mercuri triethanol ammonium lactate (Puratized Agricultural Spray) completely inhibited spore germination at concentrations

Table 5. Effectiveness of different chemicals incorporated into dextrose agar in inhibiting germination of spores of C. quercina.

Treatment applied to agar medium	Spore germination at conc. of				Germ. of check
	1000 ppm.	100 ppm.	10 ppm.	1 ppm.	
	%	%	%	%	%
Malachite green	0	0	0	0	92
Crystal violet	0	0	0	0	92
Brom cresol purple	0	32	50	56	92
Brom phenol blue	0	63	85	86	92
Phenol red	0	80	94	96	92
Thymol	0	78	88	83	97
Phenol	0	18	60	84	97
p-Nitro phenol	0	0	61	63	97
Picric acid	5	61	69	72	97
Benzoic acid	0	0	55	57	97
Gallic acid	11	51	62	86	97
Salicylic acid	0	90	95	92	95
Resorcinol	0	84	93	97	95
Potassium permanganate	32	88	92	90	95
Copper sulfate	0	82	89	93	95
Tannic acid	100	100	100	100	95
Phenyl mercuri triethanol ammonium lactate (5%)	0	0	0	0	98
Lauryl isoquinolinium bromide (20%)	0	0	62	94	98
Carosel *	0	0	16	78	98
8-hydroxy quinoline	0	0	0	0	92
Cu 8-hydroxy quinoline	0	0	0	0	92
8-hydroxy quinoline benzoate	0	0	0	0	92
8-hydroxy quinoline sulfate	0	0	0	0	92
Quinoline	0	51	78	82	91
Hydroquinone	0	20	94	91	92

Table 5. (Continued)

Treatment applied to agar medium	Spore germination at conc. of 1000 ppm.	100 ppm.	10 ppm.	1 ppm.	Germ. of check
	%	%	%	%	%
2,4,5 trichlorophenylacetate	0	24	46	77	92
U. S. Rubber 3192*	0	0	17	24	92
2,3 dichloro, 1,4 naphthoquinone (45%)	0	0	0	56	92
Tetrachloroparabenzoquinone (45%)	0	0	75	76	92
Copper (40%) zinc (10%) chromate	5	18	62	84	91
Copper (32%) zinc (15%) chromate	4	6	64	67	91
Carbide and Carbon 974*	0	0	0	69	91
2-heptadecyl glyoxalidine (54%)	0	0	82	84	91

* Experimental fungicides. Active ingredients unknown.

of 1 ppm. These chemicals were tested further to establish their respective minimum inhibitory dosages and their effect on mycelium of the pathogen. The 8-hydroxy quinoline derivatives completely inhibited germination even at concentrations of .1 ppm. 8-hydroxy quinoline sulfate was effective at .01 ppm.

It was desirable to know whether the chemicals tested were fungicidal or fungistatic in action against the pathogen. A chemical fungicidal to Chalara would offer a potential means of tree disinfestation through internal chemotherapy. Such a chemical might also be used to produce temporary immunity in healthy trees nearby infected ones and would thereby have great potentialities in the control of oak wilt, particularly in parks and residential areas. If its action was merely inhibitory, the chemical would serve only as a delaying mechanism to slow the progress of the pathogen in the host. As concentration of the chemical decreased in the tree the pathogen would again become active.

The seven chemicals most inhibitory to germination of Chalara spores were tested for fungicidal activity against Chalara mycelium. Disks of mycelium, 6 mm. in diameter, were cut from mycelial mats with a sterilized cork borer and placed in 1000, 100, 10, and 1 ppm. concentrations of the chemicals to be tested. Two disks were removed after 2, 24 and 96 hours, washed in two changes of sterile distilled water to remove chemicals and placed on potato-dextrose-agar to observe for growth.

Of the chemicals tested Malachite green, 8-hydroxy quinoline benzoate, 8-hydroxy quinoline sulfate and phenyl mercuri triethanol ammonium lactate showed the greatest fungicidal activity against Chalara mycelium (Table 6). No growth was produced by the mycelial disks exposed to 1000 ppm. of those

Table 6. Effect of exposing mycelial disks of C. quercina to different chemicals for 2, 24 and 96 hours before placing on potato-dextrose-agar.

Treatment used		Growth from disks exposed for		
Chemical	Conc.	2 hrs.	48 hrs.	96 hrs.
Malachite green	1000	-	-	-
	100	+	+	+
	10	+	+	+
	1	+	+	+
Crystal violet	1000	+	+	+
	100	+	+	+
	10	+	+	+
	1	+	+	+
8-hydroxy quinoline	1000	+	-	-
	100	+	+	+
	10	+	+	+
	1	+	+	+
Copper 8-hydroxy quinoline	1000	+	-	-
	100	+	+	-
	10	+	+	+
	1	+	+	+
8-hydroxy quinoline benzoate	1000	-	-	-
	100	+	+	-
	10	+	+	+
	1	+	+	+
8-hydroxy quinoline sulfate	1000	-	-	-
	100	+	-	-
	10	+	+	+
	1	+	+	+
Phenyl mercuri triethanol ammonium lactate	1000	-	-	-
	100	+	-	-
	10	+	+	+
	1	+	+	+

+ = Growth.

- = No growth.

chemicals for 2 hours. After 24 hours exposure to 1000 ppm. of the chemicals only the disks soaked in crystal violet grew. Even after 96 hours exposure to 1000 ppm. of crystal violet no fungicidal action was apparent. 8-hydroxy quinoline sulfate and phenyl mercuri triethanol ammonium lactate showed the greatest fungicidal activity against Chalara mycelium. Fungus disks were killed by exposure to 1000 and 100 ppm. of these chemicals for 24 hours. Copper 8-hydroxy quinoline and 8-hydroxy quinoline benzoate were toxic at 100 ppm. after 96 hours.

The possibility existed that failure to grow was due to retention of chemicals in the mycelial disks. If so, it would be expected that such water soluble chemicals would diffuse into the medium after a few days in quantity sufficient to inhibit growth. To test for such inhibitory action the treated disks that showed no growth after 10 days were transferred to fresh potato-dextrose-agar slants. Untreated disks of mycelium were placed on the area previously occupied by the treated disks. Treated disks failed to grow on the fresh medium while untreated disks grew on all of the surfaces formerly occupied by treated disks except those on which disks exposed to Puratized for 24 and 96 hours had been placed.

MODE OF SYMPTOM INDUCTION

Spread of the pathogen through the host by movement of conidia in the transpiration stream and induction of wilting by a metabolic product toxic to oaks would explain the rapid progress of oak wilt through a tree. The rapid production of conidia by the pathogen and their movement through host vessels has been demonstrated.

To test for the production of metabolic products which would induce wilting, the organism was grown in liquid culture on a modified Richard's solution which contained asparagine and yeast extract. Culture filtrates were obtained by passing the medium through a Büchner funnel and forcing the filtrate through a Seitz bacteriological filter to remove any Chalara particles or contaminating organisms. Dilutions were prepared by the addition of definite quantities of the filtrate to Erlenmeyer flasks containing measured amounts of sterile distilled water.

Preliminary tests were conducted on Bonny Best tomato cuttings. Plants were grown to a height of 5 to 6 inches at a temperature of about 80° F. Plants were cut near the groundline with a razor blade, recut under water and placed in the solution to be tested. In preliminary tests severe wilting of the cuttings was induced after 48 hours in 50 and 100 per cent concentrations of the filtrate. A small amount of drying of marginal lobes of leaflets was noted in control plants in uninoculated medium. Similar results were obtained using excised upper leaves from 10 to 12 inch plants.

The results of a test with 50 per cent concentration of the filtrate are shown in Table 7. After 24 hours all tomato cuttings in the toxic

Table 7. Extent of wilting in tomato cuttings after exposure for 12, 16, 24 and 48 hours to filtrates from cultures of C. quercina.

Treatment applied		Extent of wilting after			
Filtrate used	Repl. no.	12 hrs.	16 hrs.	24 hrs.	48 hrs.
Culture 50%	1	+	++	+++	+++
	2	++	+++	+++	+++
	3		+	++	+++
	4		+	++	+++
Medium 50%	1				
	2				
	3				
	4				
Culture 50%	5		+	++	+++
	6	++	+++	+++	+++
	7		+	+	+++
	8		+	++	+++
Medium 50%	5				+
	6				
	7				
	8				

\+ = marginal lobes of leaflets dry and curled.

\+ + = entire periphery of leaflets dry and curled.

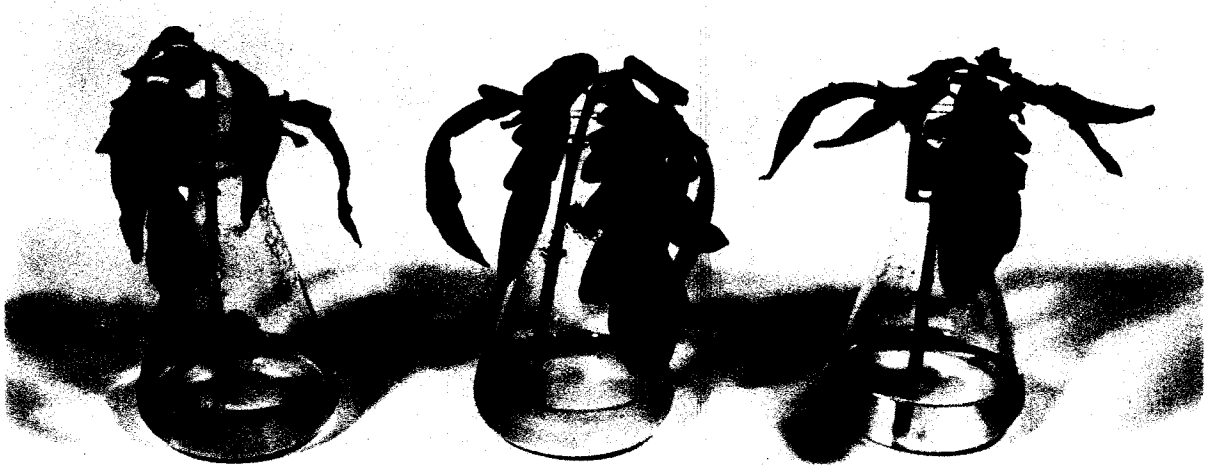
\+ + + = more than one-half of leaflet surface dried. Necrotic spots over blade.

filtrate showed symptoms of wilting (Figure 14) and after 36 hours more than 50 per cent of the leaf area had become dry and wrinkled.

To test for possible plugging of the lower stem end the cuttings were removed from the filtrate after 48 hours, recut under water and placed in distilled water. None of the wilted plants recovered. Check plants treated similarly were still moderately turgid after 108 hours. Free-hand stem sections failed to show any indications of vascular plugging in the wilted plants. Bacteria were numerous in stems of the check plants and both cortical and vascular necrosis more extensive. It was also noted that control cuttings developed roots abundantly when recut and transferred to distilled water. Roots did not develop on the cuttings that had been exposed to the filtrate.

Dilutions of the filtrate ranging from 50 per cent down to 3 per cent were tested for toxicity to tomato cuttings. Severe wilting was induced within 48 hours in concentrations down to 12½ per cent and slight marginal paling and drying of leaflets was induced at 3 per cent concentration. Wilting was induced with filtrates from cultures varying in age from 15 to 90 days.

Oak cuttings placed in the filtrate reacted similarly to greenhouse inoculated oaks. Severe wilting of young red oak cuttings was induced within 24 hours in a 50 per cent dilution of filtrate (Figure 15). Wilting was general with cupping and curling of leaves. After 70 hours large necrotic areas had developed between the veins (Figure 16). Bur and red oak cuttings showed slight signs of water loss after 24 hours, and after 120 hours were dry and papery and showed color zonation typical of that on leaves of greenhouse inoculated plants (Figure 17).



A B C

Fig. 14. Tomato cuttings showing marginal wilting of leaflets after 24 hours exposure to 50 per cent concentrations of C. quercina culture filtrate (A and C). Control cutting in center (B).

A B

Fig. 15. Red oak cuttings after 24 hours in culture filtrate (A) and uninoculated medium filtrate (B).

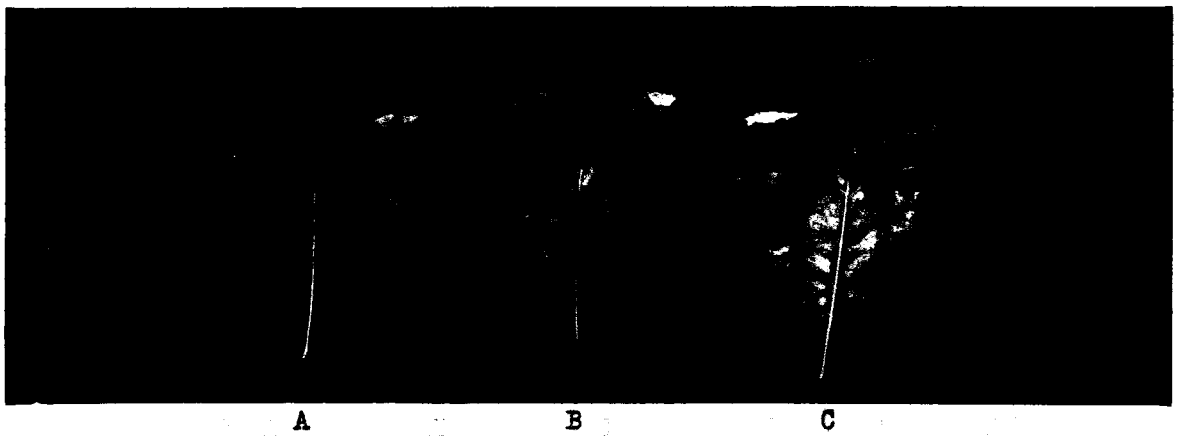


Fig. 16. Red oak leaves from young cuttings after exposure for 70 hours to uninoculated medium filtrate (A) and culture filtrate (B and C).

Fig. 17. Red and bur oak leaves from cuttings exposed for 120 hours to C. quercina culture filtrate (B and C) and uninoculated medium filtrate (A and D).

For further experimentation on induced wilting of cuttings, a controlled temperature and humidity room would be necessary. Time and severity of wilting are apparently both dependent on transpiration rate and those conditions have of necessity varied with the weather in these preliminary experiments.

CONTROL

Although sanitation is usually more effective in control of canker diseases than of wilts, studies were undertaken to determine whether (1) infested areas could be disinfested by removal of diseased trees and (2) infected trees could be saved by removal of diseased branches. A series of plots established by Dr. S. M. Dietz were carefully surveyed for infection and records were taken on the progress of the disease before and after removal of diseased members.

Eradication experiments of three types were conducted. (1) In one area of heavy infestation all dead and diseased oaks and all living oaks within a fifty foot perimeter were cut in 1944. All branches, small twigs and leaves were piled and burned and the stumps stripped of bark. Logs and large limbs were sawed and used for firewood. No further spread was observed in this plot through 1947 (Table 8, Plot 2).

(2) In a second plot all dead and diseased trees and all living red oaks within a fifty foot perimeter were cut and treated as in plot 2. Living white and bur oaks were not removed from the plot. As can be seen from Table 8, Plot 1, trees continued to become infected in that area and were killed in large numbers in 1947.

(3) Two groups of plots, separated by a 1/2 to 3/4 mile wide valley, were conveniently situated for a field comparison of the effect of eradication and were handled as follows. Spread was allowed to occur naturally in the 13 plots on one side of the valley from 1944 through the summer of 1945. In the winter of 1945-46 all dead and diseased trees were removed and disposed

Table 8. Effect of eradication of infected oaks on incidence of disease in field plots at Dolliver State Park.

Plot no.	No. dead trees 1944	No. wilting trees 1944	1945	1946	1947	Date trees in plot were cut
1	37	0	4	2	10	1-2-3/44
2	16	0	0	0	0	"
20	6	0	0	0	0	"
	8A.					
3	8	3	0	0	0	Winter '45-'46
4	9	0	3	2	1	"
5	13	0	2	1	0	"
6	6	0	1	0	0	"
7	2	0	1	0	0	"
8	8	0	0	0	0	"
9	5	0	0	0	0	"
10	4	0	0	0	0	"
11	4	0	1	2	2	"
12	6	2	4	0	0	"
18	4	1	1	0	1	"
19	2	1	0	0	0	"
22	10	1	1	0	0	"
Totals		8	14	5	4	
	8B.					
13	1	2	3	3	2	11-12/46
14	1	0	1	1	0	"
15	1	0	0	0	0	"
16	11	1	1	2	2	"
17	1	1	0	0	0	"
21	1	0	0	1	2	"
23	3	0	1	1	0	"
24	7	2	4	0	0	"
25	9	3	0	0	1	"
26	0	1	0	2	1	"
27	1	0	0	0	0	"
28	17	3	2	3	0	"
Totals		13	12	13	8	

of as in plots 1 and 2. A reduction was observed in the number of infected trees in these plots during the two seasons following cutting, Table 8A.

In the second group of plots, spread was allowed to occur without interference through the summer of 1946. All dead and diseased trees were removed during November and December of 1946. A reduction in the incidence of infection was noted in 1947, Table 8B.

From these data it appears that oak wilt may be controlled by thorough eradication of dead and diseased oaks and apparently healthy oaks within the immediate vicinity. Failure to eradicate the organism from plot 1, and subsequent spread, was probably due to mild infection of white oaks which went unnoticed in the removal of diseased trees. Removal of dead and diseased trees alone was apparently effective in reducing the spread of oak wilt. In all cases the incidence of infection was decreased by removal of dead and diseased trees.

The general distribution of the pathogen in infected red oaks precludes the use of pruning as a means of saving diseased trees of that group. However, oak wilt progresses slowly in white and bur oaks and is apparently often confined locally within the tree. If such were the case, removal of infected portions might offer a satisfactory means of saving infected white oak trees. When the distribution of symptoms was favorable on naturally infected white oaks in field plots, diseased portions of the tree were removed at points 4 to 6 feet back of the innermost symptoms. In a few cases as much as 15 or 20 feet of the top of white oaks was removed.

As shown in Table 9 the results from pruning were variable. Apparently the organism was successfully eradicated from trees 2, 7 and 9 although two prunings were necessary on tree 9. The remaining trees reacted variously in

Table 9. Effectiveness of pruning as a means of eradicating C. quercina from locally infected white oaks.

Size of trees	Date 1st pruned	Chalara isolated	Date symptoms recurred	Chalara reisolated	Remarks
(1) 6"	6-20-45	Yes	7-15-45	Yes	General symptoms Cut 7-15-45
(2) 9"	6-20-45	Yes	7-18-46	No	No further symptoms
(3) 11"	6-20-45	Yes	6-20-46 7-18-46 7-12-47	Yes Yes Yes	 General symptoms Tree cut 7-12-47
(4) 9"	6-20-45	Yes	6-19-46 9-18-47	Yes Yes	 Single branch, pruned
(5) 7" *	6-21-46	Yes	7-12-47	Yes	General symptoms. Cut 7-12-47
(6) 10"	7-10-46	Yes	7-12-47	Yes	General symptoms. Cut 7-12-47
(7) 10" *	7-25-46	Yes			No further symptoms
(8) 10"	7-13-46	Yes	7-27-46	Yes	General symptoms. Cut 7-27-46
(9) 12"	7-11-46	Yes	9-15-46	Yes	Pruned again No further symptoms

* Symptoms scattered over tree. All symptom branches removed.

regard to recurrence of symptoms. Some developed general symptoms shortly after pruning. Others showed no symptoms for over a year then developed general symptoms.

From these data it would appear that the value of pruning is variable, depending upon the relation between the extent of symptoms and the extent of the organism within the host.

DISCUSSION

Very little information has been reported previously on the life history, physiological activity and parasitic processes of Chalara quercina Henry. In the course of these investigations it has been observed that the pathogen may overwinter in infected trees and stumps. Trees of the white oak group may maintain perennial infection for several years and red oaks may often harbor the pathogen for as long as a year. Definite proof has been offered that fungus mycelium and conidia develop in xylem vessels of these hosts. The manner in which hyphae or conidia leave infected plants and are transferred to healthy plants is not known. It is entirely possible that the fungus may have a perfect stage, as yet undiscovered, which would further supplement these known reservoirs of inoculum or which may be the chief means of dissemination.

Greenhouse studies indicate that the fungus cannot establish itself in uninjured stem or leaf tissue. No successful inoculations were obtained without first providing a wound entrance for the pathogen. Once established in the host, however, the organism spreads rapidly throughout the tree. The abundant production of small conidia provides a means of rapid spread in the translocation stream. Conidia are produced most abundantly at temperatures of 20 to 28° C. (68 to 82° F.), a temperature range that prevails during the greater part of each summer day in June, July and August in this region (Table 10).

Table 10. Mean monthly temperatures (degrees F.) at Ames, Iowa, 1944-47.

	May	June	July	August	Sept.	Oct.
1944	64.6	71.7	72.6	71.8	64.3	53.8
1945	55.1	64.1	72.1	72.5	62.9	51.8
1946	56.5	69.8	73.8	68.9	63.2	55.7
1947	55.6	66.3	72.8	80.6	67.6	61.4
Average since 1873	60	69.5	74.6	72.3	63.9	51.9

Conidial production begins almost immediately following spore germination in culture and continues at a rapid rate as the fungus develops. Conidia may be found generally distributed in xylem vessels (Figure 5) of infected trees.

Considering temperature as the chief factor limiting sporulation, the most favorable time for rapid spread of the organism would be in the latter half of June when night temperatures are at a level permitting growth and daytime temperatures seldom exceed the upper limits of the optimum range. Data on time of infection as based on expression of symptoms bear out this hypothesis (Table 11).

Table 11. Number of trees developing oak wilt symptoms in field plots at Dolliver State Park and McGregor during different months of the 1946 and 1947 growing seasons.

	May	June	July	August	September
1946	5	30	29	5	5
1947	1	12	27	10	5

During 1946 the greatest number of newly infected trees were recorded in June and July, with a few observed in May, August and September. In 1947, when the season was much later, only a few trees had developed symptoms by the end of June and the peak of wilting was in July.

There is no valid reason for questioning that the conidia are carried in the transpiration stream since they were readily passed through red and white oak twigs under suction. The vessels of white oaks are smaller than those of red oaks, and more occluded with tyloses and other inclusions. Therefore, it is possible that these anatomical features may impede the movement of Chalara spores in white oak and in part contribute to the slower, more restricted, invasion of this type of oak.

The pathogen, when grown in liquid culture, produces certain metabolic products that are chemically stable and capable of inducing wilt symptoms typical of those associated with the disease. These toxic substances are not specific for oak since they also induce wilting of tomato cuttings in a similar fashion. The symptoms produced by the toxic products are so similar to the ordinary symptoms associated with oak wilt that there can be little doubt that the toxic substances play an important role in disease production. Red, white and bur oak cuttings all reacted to the filtrate to produce symptoms typical of greenhouse inoculated oaks. It is reasonable to assume, therefore, that the resistance in white oaks may be more appropriately attributed to restriction of invasion and growth of the fungus than to specificity of or immunity from the toxin.

Control of a tree disease such as oak wilt presents a tremendous problem. The sanitary measures which provided satisfactory disease control were entirely too drastic to be of practical value for large-scale

control efforts. Pruning of mildly infected white oaks may offer a means of saving individual trees. Uniformly good results from pruning elms with Dutch elm disease have been reported (55). Successful eradication of the pathogen from white oaks by pruning would depend on the reliability of symptoms as an indication of the extent of the pathogen's distribution within the tree. Preliminary results indicate that the organism is often restricted to the region of symptoms in white oaks. The possibility of saving these trees by pruning merits further investigation.

In general, attempts to control lethal tree diseases such as oak wilt by sanitary measures have at best only succeeded in retarding the diseases. In recent years the trend in plant disease control has been toward various types of chemical treatment. The preliminary tests on chemical inhibition are the first step in investigations toward combatting oak wilt through internal chemotherapy. An ideal chemical for such treatment would be water soluble and would possess a toxicity differential for fungus and oak great enough to permit eradication of the pathogen without seriously injuring the host. Such a chemical could be injected directly into the bole or put into the soil water so as to be taken up in the transpiration stream (55). Even though a chemical is not found with a toxicity differential great enough to permit eradication of the pathogen without damage to the host, a good disinfestation process would be of value. Such chemically disinfested trees could then be felled and worked up without danger of spreading the pathogen to surrounding trees.

SUMMARY

Chalara quercina was observed to overwinter commonly on white and bur oak trees, in stumps of diseased trees that had been removed, and occasionally on red oak trees that were infected late in the season.

The disease spread in a pattern not typical of wind blown spore or insect dissemination. Oak wilt usually spread from an infection locus to the nearest healthy trees.

Data on percentage infection of red and white oak species showed little difference in susceptibility to infection. However, data on destruction of species of oak showed much greater losses in trees of the red oak group than in trees of the white oak group.

No difference was observed in the incidence of infection of different sizes of trees. The number of infected trees of a given size was proportional to the total number of trees of that size in an area.

No host specificity was observed in cross-inoculation tests with isolates from different species of oaks. Isolates from each species produced typical disease symptoms when inoculated into other species. Some variation in cultural growth characteristics of the different isolates was observed.

C. quercina was isolated from all parts of diseased trees except the acorn. In histological preparations the fungus was observed in xylem vessels of leaf midribs, petioles and stems. Conidia were observed in host tracheae, and conidia in water suspension passed readily through red and white oak stem sections.

Isolates of Chalara quercina grew well over a range from 16° to 28° C.

with most rapid growth at 22° to 26° C. The optimum temperature range for spore germination was 25° to 30° C. At 25° C. endogenous conidia were typically produced from germ tubes of all germinating spores, while at 30° C. more than 85 per cent of the spores formed vegetative hyphae on germination.

The pathogen made good growth on all complete nutrient media tested but grew best on potato-dextrose-agar and oatmeal agar.

Spores germinated best at 1 per cent dextrose concentration. Spore production from germinating spores tended to increase as the sugar concentration was decreased from 4 per cent to 0.1 per cent.

Light apparently had no effect on either growth or spore germination.

Optimum pH range for growth was from pH 5 to pH 7 with limits at pH 3 and pH 9.

Spore germination was completely inhibited by 1 ppm. of malachite green, crystal violet, 8-hydroxy quinoline, copper 8-hydroxy quinoline, 8-hydroxy quinoline benzoate, 8-hydroxy quinoline sulfate and phenyl mercuri triethanol ammonium lactate. Even at .1 ppm. the 8-hydroxy quinoline derivatives were completely inhibitory to spore germination.

The action of crystal violet, as indicated by tests on mycelial disks, was probably fungistatic. 8-hydroxy quinoline sulfate and phenyl mercuri triethanol ammonium lactate showed the greatest fungicidal activity against Chalara mycelium. Fungus disks were killed by 24 hour exposure to both 1000 and 100 ppm. of those compounds.

A metabolic product, which readily induced wilting of tomato and oak cuttings, was produced by C. quercina when grown on a modified Richard's solution containing asparagine and yeast extract. Symptoms on toxin-wilted oak cuttings were typical of those produced on diseased greenhouse oaks.

Eradication experiments showed that the pathogen could be removed from an area by extreme sanitary measures. Incidence of infection was reduced by removal of diseased trees from areas of infection.

Results from pruning experiments were inconsistent but indicated that white oaks might often be saved by removal of diseased parts soon after the appearance of symptoms. Diseased branches were pruned at a point 4 to 6 feet back of the symptoms.

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VITA

The author was born March 1, 1921 on a farm at McAlister, New Mexico, the youngest of two boys of John Arthur Young and Etta Sprinkle Young. Elementary and high school training was received in the McAlister Consolidated school. After graduation from high school in 1937 two years were spent at the Eastern New Mexico Junior College, Portales, New Mexico, from which an associate in arts diploma was received in 1939. The Junior and Senior years of college were spent at New Mexico A. and M. from which he was graduated in 1941 with a Bachelor of Science degree with a major in biology and minor in chemistry. He entered Iowa State College graduate college in September 1941 and under the direction of Dr. G. C. Kent completed requirements for an M.S. degree in plant pathology in December 1942. The following 3¼ years were spent in the U. S. Navy, first in training at Columbia Midshipmen's School then as a deck officer. He was released to inactive duty with the rank of Lieutenant (D) USNR May 8, 1946 after which studies toward a doctorate in plant pathology were resumed. Graduate work toward the doctorate was taken under the direction of Dr. S. M. Dietz and Dr. George L. McNew.